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By

ELIZABETH DOROTHY WUIST

A thesis presented to the faculty of the Department of
Literature, Science, and the Arts, of the University
of Michigan in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

[PHYSIOLOGICAL RESEARCHES VOL. I, NO. 3, SERIAL NO. 3. SEPTEMBER, 1913]

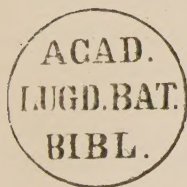
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SEX AND DEVELOPMENT OF THE GAMETOPHYTE OF *ONOCLEA STRUTHIOPTERIS*.¹

ELIZABETH DOROTHY WUIST.

ABSTRACT.²

This paper deals mainly with the question as to whether the nature of the sex organs of the gametophyte of *Onoclea struthiopteris* is predetermined, or whether this gametophyte may be induced by external conditions to produce either male or female organs exclusively, or both kinds of organs together. Both soil and solution cultures were employed, with various nutrient solutions in the latter, and with different intensities of insolation. From these experiments, extending over a period of several years, it appears that the sex of this gametophyte is not predetermined in the spore from which the plant developed, but that each prothallium is capable of forming both kinds of sex organs, either antheridia or archegonia, or both, according to the external conditions. It seems that both male and female tendencies are present as potentialities in the cells of the gametophyte and are influenced, in their visible expression, by the factors of the environment during the life history of the organism. The main results of the study follow.

(1) The gametophyte generation of *Onoclea struthiopteris* is shown in this study to be either monoecious or apparently dioecious, according to developmental stage and environmental conditions. In younger cultures in soil five per cent. of all the prothallia were monoecious; in older cultures, fifteen per cent. were monoecious.

(2) Ninety per cent. of the prothallia which originally bore only female organs were induced later to produce antheridia and thus become monoecious, by placing them under favorable conditions of nutrition.

(3) Five per cent. of the individuals which originally bore only male organs were induced later to produce archegonia and thus to become monoecious, by placing them under conditions of nutrition which were favorable to further growth and development.

(4) Young archegonia were formed after fertilization had taken place.

(5) Contrary to the observations of Prantl, the position of the prothallia in the soil cultures did not influence the kind of sex organs developed.

¹ Contribution No. 138 from the Botanical Department of the University of Michigan.

² The manuscript of this paper was received April 15, 1913. This abstract was preprinted without change from these types, and was issued as Physiological Researches Preliminary Abstracts, vol. 1, no. 3, August, 1913.

(6) The male tendency appeared to be latent in all parts of the apparently female gametophyte, as shown by the later development of antheridia from various cells and regions.

(7) Both apparently male and apparently female prothallia were obtained in cultures of Prantl's solution from which nitrogen had been omitted.

(8) Rubidium hydrate, apparently by hindering growth, increased monoecism.

(9) Good growth and development of these gametophytes occurred in solutions without either sodium or calcium.

(10) Adventitious outgrowths were developed by the prothallia, in response to unfavorable environmental conditions.

(11) Rhizoids and reproductive organs were frequently borne upon the dorsal surfaces of the prothallia.

INTRODUCTION.

The following investigation was undertaken with the view of determining whether the gametophyte of *Onoclea struthiopteris* is always dioecious and, if it is not, what may be the environmental conditions necessary for the development of dioecious and monoecious prothallia. Later, this was extended to an experimental study of the gametophyte generation. Here the physiological phase, rather than the morphological, was emphasized, the latter being considered only so far as it had direct bearing upon the former.

The experimental work of this paper was done during the past four years, in the Botanical Laboratory of the University of Michigan. My thanks are due Dr. C. H. Kauffman, who suggested the problem and under whose guidance and inspiration the work has been carried on. I am also indebted to Professor F. C. Newcombe for many valuable suggestions and criticisms.

HISTORICAL.

The development of the gametophyte generation of ferns has, until recently, been studied more extensively from the morphological than from the physiological point of view. A historical review of the earliest literature relating to the morphology is given by Hofmeister.³ In more recent years the literature has become very extensive; but, as it is concerned with the morphology of the prothallia of a number of genera, mention will be made here only of such work as has a direct bearing upon this study. Campbell⁴ is the only one who has worked out in detail the morphology of the gametophyte and sporophyte generations of *Onoclea struthiopteris*.

³ Hofmeister, W., Higher Cryptogamia. Translated by Frederick Currey. London. 1862.

⁴ Campbell, D.H., The development of the ostrich fern. Mem. Boston Soc. Nat. Hist. 4, No. 2. 1887.

Brief studies of the conditions controlling the sex of the gametophyte generation of this fern have been published by Mottier⁵ and myself;⁶ while Terletzki [84] and Milde [53] confined their study to the sporophyte generation.

The physiological study of the development of the gametophyte began with a study of the germination of the spores. The literature relating to this phase of study has been so recently summarized by Heald [98], Shultz [02], Life [07], Laage [07], Burgerstein [08] and Fischer [12], that reference need only be made to these summaries.

The first work upon the physiological development of these organisms was a study of the bilaterality of the prothallia, and the effect of light upon the formation of the reproductive organs. Leitgeb⁷ demonstrated, by cultivating fern gametophytes on a nutritive solution, that the bilaterality of the prothallia was due to the effect of light, not gravity, as had been thought by some of the earlier writers. He found by altering the illumination that it was possible to produce changes in both sides of the prothallia. He also found that the reproductive organs were always borne on the shaded side. Two years later, Prantl,⁸ working with *Aneimia phyllitidis*, *Polypodium vulgare*, *Osmunda regalis* and *Aspidium filix-mas*, verified the results of Leitgeb in regard to the bilaterality of the prothallia and the position of the sexual organs. In addition, he reported that strong light tended to produce only archegonia and weak light only antheridia. Klebs,⁹ with strong light, obtained large heart-shaped prothallia bearing both kinds of organs. Under the action of weak light the peripheral cells of his prothallia grew out into filaments, the ends of which in some cases developed prothallia when placed in strong light. He also showed that, by reducing the light intensity, vegetative growth could be prolonged and the reproductive organs, both male and female, repressed. Heim¹⁰ carried this line of investigation still further, experimenting with strong and weak white light, and with the yellow, blue and violet rays of the spectrum. His results with *Aneimia phyllitidis* were as follows: Strong light produced heart-shaped prothallia bearing archegonia and antheridia. Less intense light produced elongated prothallia, also bearing archegonia and antheridia. In yellow light long narrow prothallia developed, bearing antheridia but no archegonia. The blue and violet rays checked growth

⁵ Mottier, David, Note on the sex of the gametophyte of *Onoclea struthiopteris*. Bot. Gaz. 50, 209. 1910.

⁶ Wuist, Elizabeth D., The physiological conditions for the development of monoecious prothallia in *Onoclea struthiopteris*. Bot. Gaz. 49: 216. 1910.

⁷ Leitgeb, H., Ueber Bilateralität der Prothallien. Flora 60: 174. 1877.

⁸ Prantl, K., Ueber den Einfluss des Lichtes auf die Bilateralität der Farnprothallien. Bot. Zeitg. 37: 713, 719. 1879.

⁹ Klebs, G., Ueber den Einfluss des Lichtes auf die Fortpflanzung der Gewächse. Biol. Centralbl. 13: 641. 1893.

¹⁰ Heim, Carl, Untersuchungen über Farnprothallien. Flora 82: 328. 1896.

and produced only adventitious prothallia. Life,¹¹ with cultures of *Dicksonia aprifolia*, *Aneimia phyllitidis* and some other ferns, verified in the main the results of former work. His results indicated that light intensity affected the form of the prothallia and the formation of reproductive organs. Strong light favored the development of heart-shaped gametophytes in all the ferns studied by him except *Aneimia*, and it also favored the production of both antheridia and archegonia except in *Alsophila pruinata*, where only archegonia were produced. Weak light produced "filamentous or ribbon-like prothallia." It inhibited the development of archegonia and favored the production of antheridia. Life's experiments also indicated that young stages of *Alsophila pruinata*, *Aneimia phyllitidis* and *Gymnogramme* are strongly and positively heliotropic. Peirce¹² found that the prothallia of *Gymnogramme* grown from spores in Knop's solution, on a klinostat, were dorsiventral. He believes that the direction of illumination and not mechanical resistance determines the direction of growth of the germ tube and of the first cell divisions. He also believes that light determines which side of the prothallus shall develop the rhizoids and reproductive organs. Peirce's experiments showed that when the illumination was approximately equal and sufficient on all sides, the rhizoids and sexual organs developed on both sides of the prothallia. Furthermore, insufficient light suppressed the development of reproductive organs in this fern.

The effect of nutritive conditions upon the development of prothallia and reproductive organs was first investigated by Prantl.¹³ He concluded that the prothallia in crowded regions of soil cultures were "ameristic" and produced only antheridia, while in less crowded regions "meristic" prothallia developed and bore only archegonia. From spore cultures of *Polypodium vulgare*, *Aspidium filix-mas*, *Ceratopteris thalictroides* and *Osmunda regalis*, on nutrient solution, he reports that the prothallia in cultures without nitrogen remained "ameristic" and bore only antheridia, while those in cultures of the full solution were "meristic" and bore archegonia. From these observations Prantl drew the general conclusions that the development of the prothallia, depending on the food supply, and the separation of the reproductive organs, go hand in hand; that "ameristic" prothallia develop antheridia while "meristic" prothallia develop archegonia; that lack of nitrogen hinders the development of a meristem, and that, therefore, an insufficient nitrogen supply may account for the fact that thickly grouped prothallia bear only antheridia.

¹¹ Life, A.C., Effect of light upon the germination of spores and gametophytes of ferns. Missouri Bot. Garden Report 18: 100. 1907.

¹² Peirce, G.J., Studies of irritability in plants. Ann. Bot. 20: 449. 1906.

¹³ Prantl, K., Beobachtung über die Ernährung der Farnprothallien und die Verteilung der Sexualorgane. Bot. Zeitg. 39: 753. 1881.

EXPERIMENTAL

A. TECHNIQUE.

Few details of technique which were applicable to my work could be gleaned from earlier sources; therefore the cultural methods used in this experimental work have been the gradual outgrowth of my own experience. It is only by experience that the value of careful and painstaking attention to details of technique can be understood and appreciated.

1. Materials.

The spores used in the early part of the work were collected from two sources: from sporophytes growing on lawns about Ann Arbor, Michigan, and from sporophytes growing under natural environmental conditions in woods about two and a half miles from that city. A careful comparative study, under various conditions, was made of cultures with spores from the two sources, to see whether any differences might be noticed, either in the germination of the spores, or in the development of the prothallia and their reproductive organs. No differences were observed, however, and only spores collected from the lawns were used throughout the remainder of the work.

Collections of fertile fronds were made about the first of October of each year. They were allowed to dry by exposing them before an open window, for from a week to ten days, depending upon the condition of the fronds when collected and that of the weather. For future use the fronds were wrapped in oiled paper and preserved in a covered pasteboard box in a cool dry place. The spores were prepared for sowing by shaking a frond over paper or a clean glass plate and the sporangia obtained were crushed with a scalpel or a microscope slide, care being used not to crush the spores. The crushed mass was then rubbed through a sieve which separated the spores from the remains of the sporangia. A sieve for this purpose was constructed by stretching four thicknesses of silk bolting-cloth (obtained from a flour mill) in a small embroidery hoop. By adding or removing a thickness of cloth, the grade of the sieve could be readily adjusted.

2. Soil Cultures.

The leaf mould used in the soil cultures was taken from a wood in which no ferns grew. New flat pots or pans, $5\frac{1}{2}$ inches in diameter and 2 inches deep, were sterilized dry for from ten to fifteen minutes, at a temperature of 200° C. The pots were then filled to within an inch of their tops with leaf mould of medium fineness. This was carefully packed and smoothed, and a half inch layer of very fine soil was then spread over and pressed down to give a firm, smooth surface. Here great care was needed; if the

soil layer was too fine or too firmly packed, there was a tendency, in the subsequent alternate wetting and drying, for the surface to harden and crack. After the contents had been saturated with distilled water, the pots were covered with glass plates and sterilized with steam for about ten minutes, at a temperature of 120° C. It was found that the prothallia did not thrive as well upon leaf mould which had been sterilized for a longer time. As soon as the pots were cooled, the contents were again saturated with sterilized distilled water, and the spores were sown.

Of the various methods tried for sowing the spores, the most successful was the following. A mass of spores was taken on the point of a broad-bladed scalpel, and the instrument was moved over the pot, about half an inch above the soil surface, while the spores were gently blown upon. In this manner the spores were fairly evenly distributed.

The cultures were covered with sterilized glass plates and placed in the greenhouse, where they were exposed to direct sunlight. The first watering was done from below, thus preventing the spores from being washed into masses. Sterilized distilled water was placed in glazed saucers in which the pots stood, but an excess of water was never allowed to stand in the saucers. After germination began, watering was accomplished from above by allowing water to flow slowly down the inner surface of the pot wall. The amount of moisture present was always carefully regulated and the cultures were never watered unless the appearance of the soil indicated that this was necessary. During germination and the early development of the prothallia, it was necessary to water the cultures every day; later, when the prothallia were more fully developed, it was found best to allow the cultures to become more nearly dry at times. The moisture conditions were also regulated by raising and lowering the glass covers. On dark, cloudy days, when the temperature of the greenhouse was relatively high and the atmosphere moist, the covers were raised about one-half inch by inserting pieces of wood between them and the rims of the pots. On bright days, with lower temperature and humidity, the covers were lowered. Some workers have successfully regulated the supply of moisture to such cultures by sinking the pots in boxes of wet sand or sphagnum moss, but in all the cultures of prothallia for my own experimental work, as well as for class use, the best results have been obtained by keeping the pots themselves as dry as possible. This could best be done by keeping the contents of the pots from being too moist, and leaving the pots exposed to the air and sunlight. Very little trouble was experienced with algae or fungi. If such organisms appeared on the outer or inner surfaces of the pots the latter were cleaned at once by swabbing the surface with cotton saturated with alcohol. If fungi appeared in the cultures themselves, the infested

part was immediately removed with a sterilized, sharp-pointed scalpel or forceps. Certain chemical solutions have been used by other workers to free such cultures from algae and fungi. Miss Twiss,¹⁴ working with cultures of *Aneimia* and *Lygodium*, used potassium permanganate solution to prevent growth of algae. She found a solution containing 0.015 g. of potassium permanganate in 3.5 liters of water very successful for this purpose. It did not seem best, however, in this experimental work, to introduce any such substance, which might possibly affect the final results.

3. Solution Cultures.

In preparing the solution cultures, 26 cc. of a nutrient solution, to which had been added a drop of one per cent. solution of ferric chloride, was poured into a small, sterilized glass capsule. The spores were sown upon the solution, and the cultures thus prepared were placed in the greenhouse, under the same light and temperature conditions as prevailed for the soil cultures. Once a week the prothallia were transferred to fresh solutions, this being done by simply lifting them, with a sterilized seeker, from the old to the new solution. Care had to be exercised not to immerse the prothallia in the solution, for if this accidentally occurred it required considerable skill to make them remain on the surface afterwards; however, with older prothallia this could sometimes be accomplished by quickly drying the upper surfaces with filter paper.

A liter of each nutrient solution was made up, from Kahlbaum's pure chemicals, and these solutions were kept in flasks. In the beginning of the work each solution was carefully sterilized before it was used; it was decided later, from experimental evidence, that the cultures did better on unsterilized solutions—probably because of chemical changes caused by heating. This was especially true of solutions containing ammonium nitrate. As the stock flasks were kept well stoppered with cotton and in a clean place, and as care was used in opening them, little trouble was experienced with contamination by growths of algae or fungi.

The formulas used in making up the nutrient solutions here employed are given below.

I. Beijerinck's Solution.

NH_4NO_3	0.5 g.
KH_2PO_4	0.2 g.
MgSO_4	0.2 g.
CaCl_2	0.1 g.
FeCl_3	trace.
Distilled water.....	1000 cc.

II. Knop's Solution.

MgSO_4	0.25 g.
$\text{Ca}(\text{NO}_3)_2$	1.00 g.
K_2HPO_4	0.25 g.
KCl	0.12 g.
FeCl_3	trace.
Distilled water.....	1000 cc.

¹⁴ Twiss, Edith M., The prothallia of *Aneimia* and *Lygodium*. Bot. Gaz. 49: 168. 1910.

III. Sachs' Solution.

KNO ₃	1 g.
NaCl.....	0.5 g.
CaSO ₄	0.5 g.
MgSO ₄	0.5 g.
CaHFO ₄	0.5 g.
Distilled water.....	1000 cc.

IV. Prantl's Solution. (Prantl [81].)

K ₂ SO ₄	0.7 g.
NaCl.....	0.23 g.
CaSO ₄	0.7 g.
MgSO ₄	0.5 g.
Na ₃ PO ₄	0.5 g.
NH ₄ NO ₃ solution, 0.064	
per cent.....	20 cc.

V. Rubidium Solution. (original)

RbOH.....	0.2 g.
Ca(NO ₃) ₂	0.5 g.
KH ₂ PO ₄	0.5 g.
MgSO ₄	0.5 g.
FeCl ₃	trace.
Distilled water.....	1000 cc.

The following modified series of the above solutions were also used; items not mentioned are the same as in the corresponding unmodified solution.

Knop's Solution.

II a RbOH, 0.2 g. added.
II b MgSO ₄ , 2.5 g.; KH ₂ PO ₄ , 2.5 g.; KCl, 1.2 g.
II c KH ₂ PO ₄ , 0.37 g.
II d Ca(NO ₃) ₂ , 0.50 g.; MgSO ₄ , 0.74 g.; KH ₂ PO ₄ , 2.00 g.
II e Ca(NO ₃) ₂ , 0.50 g.; MgSO ₄ , 2.00 g.; KH ₂ PO ₄ , 0.74 g.
II f Ca(NO ₃) ₂ , 0.74 g.; MgSO ₄ , 0.50 g.; KH ₂ PO ₄ , 2.00 g.
II g MgSO ₄ , 1.00 g.; KH ₂ PO ₄ , 1.00 g.
II h Ca(NO ₃) ₂ , 1.00 g.; MgSO ₄ , 1.00 g.; KH ₂ PO ₄ , 1.00 g.

Sachs' Solution.

III a KNO ₃ , 5.00 g.

Prantl's Solution.

IV a NH ₄ NO ₃ omitted.
IV b NaCl and Na ₃ PO ₄ omitted.
IV c NaCl omitted.
IV d Na ₃ PO ₄ omitted.
IV e NaCl, 2.3 g.; NH ₄ NO ₃ , 6.4 g.
IV f NH ₄ NO ₃ omitted; NaCl, 2.3 g.
IV g NaCl and Na ₃ PO ₄ omitted; NH ₄ NO ₃ , 6.4 g.
IV h NaCl omitted; NH ₄ NO ₃ , 6.4 g.
IV i Na ₃ PO ₄ omitted; NaCl, 2.3 g.; NH ₄ NO ₃ , 6.4 g.

Rubidium Solution.

V a Ca(NO ₃) ₂ omitted.
V b RbOH, 0.5 g.; Ca(NO ₃) ₂ , 0.8 g.; KH ₂ PO ₄ , 0.8 g.
V c RbOH, 0.6 g.
V d RbOH, 0.6 g.; KH ₂ PO ₄ , 3. g.
V e RbOH, 1.00 g.; Ca(NO ₃) ₂ , 1.2 g.; KH ₂ PO ₄ , 1.2 g.; MgSO ₄ , 1.2 g.
V f RbOH, 2.5 g.; Ca(NO ₃) ₂ , 1.1 g.; KH ₂ PO ₄ , 2.5 g.; MgSO ₄ , 2.5 g.

Cultures were also made by sowing the spores upon sterilized distilled water in small glass capsules and exposing them to direct sunlight in the greenhouse. Ten days later, when the prothallia had attained a growth of ten cells, one hundred were transferred, with sterilized seeker and dissecting microscope, to each of the following nutrient solutions, as above described, and modifications of these as shown below.

- | | |
|---------------------------------------|--|
| I. Beijernick's Solution, unmodified. | II. Knop's Solution, unmodified. |
| I a NH_4NO_3 omitted. | II a RbOH , 0.2 g. added. |
| I b KH_2PO_4 omitted. | |
| I c CaCl_2 omitted. | |
| III. Sachs' Solution, unmodified. | IV. Prantl's Solution, unmodified. |
| III a KNO_3 , 0.5 g. | IV a NH_4NO_3 omitted. |
| | IV c NaCl omitted. |
| V. Rubidium Solution, unmodified. | VI. Birner and Lucanus' Solution,
modified. ¹⁵ |
| | MgSO_40.5 g. |
| | $\text{Ca}(\text{NO}_3)_2$1.5 g. |
| | KH_2PO_41.0 g. |
| | Fe Cl_3trace. |
| | Distilled water.....1000 cc. |

These cultures were placed under the same conditions of light and temperature, and were given the same care and attention as the older solution cultures. By thus germinating the spores upon distilled water, and transferring the young prothallia to nutrient solutions, the number placed upon each solution was definitely known. At the same time, even the smallest fragments of sporangia were eliminated from the cultures. A question might be raised in regard to a possible shock, which prothallia might receive when transferred from distilled water to nutrient solutions, but the results obtained with these transfer cultures were practically the same as when the spores were sown directly upon the nutrient solutions, and it does not seem necessary to lay stress upon this point.

B. CULTURE STUDIES.

The germination of spores and the development of prothallia and reproductive organs will now be considered. The study of the germination of the spores, in both soil and solution cultures, was emphasized only as a means of determining the most favorable physiological conditions for rapid germination.

¹⁵ Birner and Lucanus' [66] solution contains, unmodified, 1.1 g. of ferric phosphate, but a trace of the chloride is here substituted for the phosphate. With repeated trials the chloride proved much more satisfactory.

1. Soil Cultures.

Germination began in the soil cultures from four to six days after the spores were sown. Growth was rapid but not uniform for the different prothallia, some developing more rapidly than others from the very beginning. Reproductive organs appeared about six to eight weeks after the spores

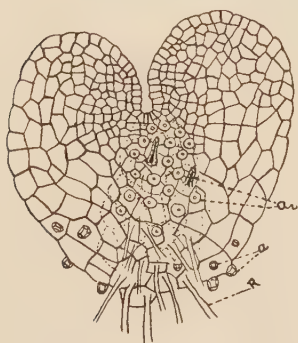


Fig. 1. Surface view of monoecious gametophyte from soil culture. *a*, antheridia; *ar*, archegonia; *R*, rhizoids.

were sown. Monoecious, as well as dioecious¹⁶ prothallia were observed. At this time, about one to five per cent. of all the prothallia were monoecious, the others seemed regularly dioecious. The monoecious prothallia were not as large as the females but were of medium size, heart-shaped, notched, and bore archegonia on a well developed meristem, and antheridia on the surface and margins of the wings. The cellular structure was apparently good and they appeared to be normal, healthy gametophytes. (Figures 1 and 2.)

Among the apparently dioecious prothallia, the females were large, distinctly

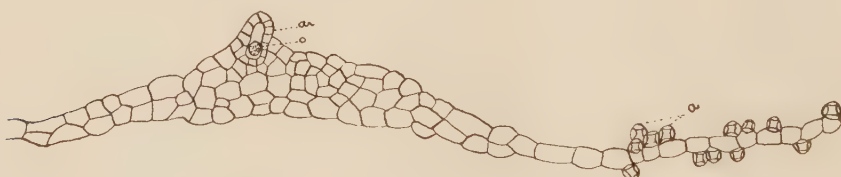


Fig. 2. Transverse section of monoecious gametophyte similar to that of Fig. 1. *a* and *ar* as in Fig. 1; *o*, oospore.

margins. These did not appear to be in a healthy condition; few chloroplasts were present in the cells. Other male prothallia were regular in shape, somewhat elongated, with a slightly heart-shaped apex; these also bore antheridia on both surfaces and along the margins (Figures 4, 5 and 6).

¹⁶ In order to avoid possible ambiguity it may be remarked that the terms *male* and *female* are employed throughout this paper in no other than a purely descriptive sense. Gametophytes which are apparently dioecious, such as have formerly been thought to produce only male or female organs, will thus be called male or female. These prothallia with but one kind of sexual organs will be termed *dioecious*, also in a purely descriptive sense. Such forms become obviously monoecious if, later, they form organs of the opposite sex.

The position of the prothallia in the cultures did not seem greatly to influence either their development or their sex, contrary to what Prantl [81] found for certain other ferns. Large, heart-shaped, female prothallia were frequently found in crowded regions, while male prothallia occurred in less crowded regions and monoecious prothallia were found throughout the cultures. Occasionally slight elevations were accidentally formed on



Fig. 3. Surface view of dioecious, female gametophyte from soil culture. *ar*, archegonia; *R*, rhizoids.

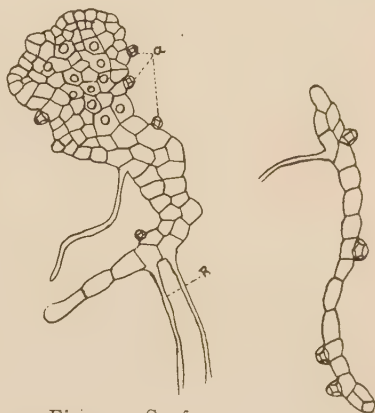


Fig. 4. Surface view of dioecious, male gametophyte from soil culture. *a*, gametophyte antheridia; *R*, rhizoids.

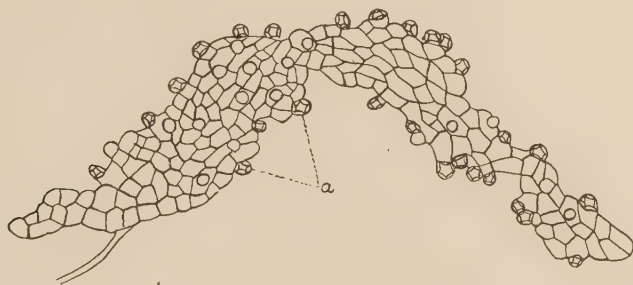


Fig. 6. Large male gametophyte from soil culture. *a*, antheridia.

the surface of the leaf mould, and upon some of these only male prothallia were found, with female prothallia crowded about the base of the elevated area. Upon others, the reverse was true, the archegonial prothallia being closely grouped on the elevation, with males scattered at the base. Likewise, in some slight depressions formed at the sides of the pots, female prothallia could be found grouped together, in others only the male form.

Twelve to sixteen weeks after the spores were sown, the percentage of monoecious prothallia in the cultures was greatly increased. Sometimes as much as fifteen to twenty per cent. of the individuals were monoecious.



Fig. 7. Outline of gametophyte neglected for a time and then rejuvenated, showing irregular outgrowths bearing antheridia. *a* antheridia; *R*, rhizoids.

This percentage nearly approaches that of the latest count (12 per cent.) given by Mottier [10]. While the majority of these monoecious prothallia were in good condition, some were not as healthy; the latter appeared to be merely older female prothallia which had become monoecious by the development of antheridia upon their surfaces and along the margins.

Some of these same cultures, from which many of the prothallia had been removed for experimental purposes, were later placed on an interior laboratory table and neglected for about two weeks. At the end of that time all the prothallia appeared dead. The pots were then placed under bell-jars near the window and kept well watered. In about a week outgrowths began to form from the margins of the old prothallia, and six to eight weeks afterward these outgrowths had developed into new prothallia of various shapes, which were sometimes monoecious and sometimes dioecious. Some of these dioecious outgrowths were filamentous in form and were covered with antheridia. Other outgrowths developed into elongated prothallia bearing archegonia, which, in turn, became monoecious, either by developing antheridia on the surface and margin or by developing adventitious shoots bearing antheridia. (Figure 7.)

2 Solution Cultures.

The time of germination, both in nutrient solutions and in sterilized distilled water, was approximately the same as that in the soil cultures. Four days after the spores were sown germination began. Slight differences in the early development of the prothallia could be noted in these cultures. In distilled water, in most instances, there was a tendency for three cells to be formed by transverse divisions; these cells not infrequently became markedly elongated before the terminal one was divided by an oblique longitudinal wall. Thus the prothallia assumed a more or less filamentous form. In the nutrient solutions of lower concentration, as a rule, only two cells were formed before longitudinal division began, the second cell being soon divided by an oblique longitudinal wall. No noticeable elongation took place in either of these two cells. In solutions of higher concentration, the division of the cells in the young gametophytes followed that occurring in solutions of less concentration, but the shape of the cells differed from those formed either in solutions of lower concentration or in distilled water by being very short and broad. Thus, the young prothallia immediately assumed a flattened form, no filamentous or protonemal stage being observable.

The following results are based upon the study of the prothallia as they developed in the nutrient solution cultures previously described. As has been remarked, no fundamental differences were noted at any time, between the prothallia developed from spores sown directly upon nutrient solutions and those transferred at early stages from sterilized distilled water to nutrient solutions. The period over which most of these observations extended, varied from twelve to sixteen weeks for each set of cultures. (Figures 8, 9, 10.)

Solution I (Beijerinck's solution, unmodified). In the younger cultures all of the prothallia were dioecious, while in the older cultures about one-third of the female prothallia became monoecious by the development of proliferations bearing antheridia. The average female was large, ranging in size from 6×3 mm. to 7×3 mm. The male prothallia were of the usual size and shapes. Well developed sporophytes were present in all the other cultures.

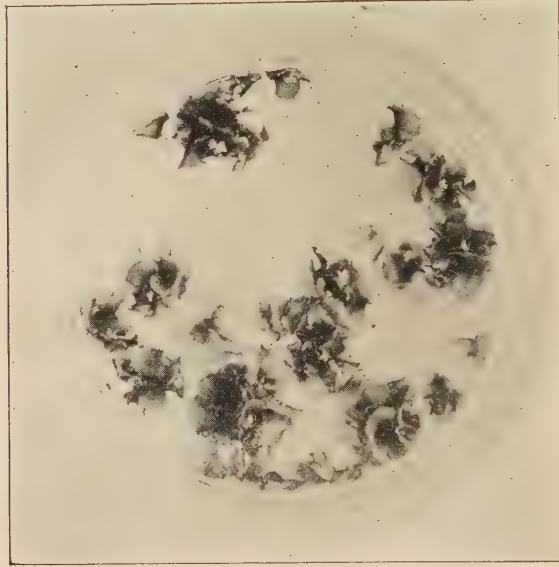


Fig. 8. Surface view of a nutrient solution culture, showing the prothallia.¹⁷

Solution Ia. No monoecious prothallia were observed. All the prothallia were small and, as in the cultures of solution IVa, about one-third of the individuals were females and two-thirds males. The female prothallia resembled those in solution IVa, in size, shape and general appearance, but were more distinctly notched and generally bore fewer archegonia.

Solution Ib. The prothallia were small, poorly developed and short-lived. Antheridia, but no archegonia were formed.

Solution Ic. All the prothallia were dioecious. The proportion of males to females, the size, shape, general appearance and development of the reproductive organs were the same as in solution I.

Solution II (Knop's solution, unmodified). Germination of the spores, and growth of prothallia were very good. In the young cultures more

¹⁷ This and the other photographic prints here employed were made by Miss Frances Dunbar.

than two-thirds of the prothallia were dioecious. The majority of these were females, heart-shaped, notched, and with well-developed meristems and numerous archegonia in various stages of development. The average size was 6.5×3 mm. However, a large number of monoecious individuals were observed in the older cultures. These resembled the females in size, shape and general appearance. Most of the males were regular in shape, elongated, with slightly heart-shaped apex. Vigorous young sporophytes were present. One of these sporophytes was kept alive five months, and developed five fronds, one of which was 2.5 cm. in length. (Figure 11.)

In some of the cultures in solution II the prothallia were never transferred to fresh solutions, but were allowed to develop upon the same solutions on which the spores had been sown. Here the average size was approximately the same as when the transfer had occurred, but the per-

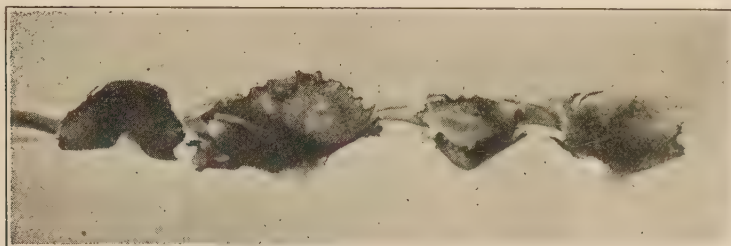


Fig. 9. Side view of portion of the same culture as is shown in Fig. 8.

centage of monoecious individuals was much larger. Of the dioecious prothallia, about two-thirds were males and one-third females.

Solution IIa. Germination of spores and early growth of prothallia were rapid; later growth was slower. Twelve weeks after the spores were sown *sixty per cent. of the prothallia were monoecious.* Among the dioecious individuals, there were more males than females. Both the monoecious and the female prothallia were heart-shaped and distinctly notched, and varied in size from 5×2 mm. to 4×2 mm. The monoecious individuals were normal and in a healthy condition. The female prothallia appeared healthy, but bore fewer archegonia than the average female of other cultures. The male prothallia were of the usual sizes and shapes. No sporophytes were observed.

Solution IIb. Both in growth and development the gametophytes closely resembled those in solution II. The average size was somewhat larger than in that solution, but the proportion of monoecious to dioecious prothallia in young and old cultures was the same. In cultures where the nutrient solution had not been changed, the prothallia were smaller than in the

corresponding culture with solution II, the average size was only 5×2.5 mm. and the percentage of monoecious individuals was the same. Sporophytes were present in all the cultures.

Solution IIc. The spores failed to germinate in either the cultures in full light or those in reduced light.

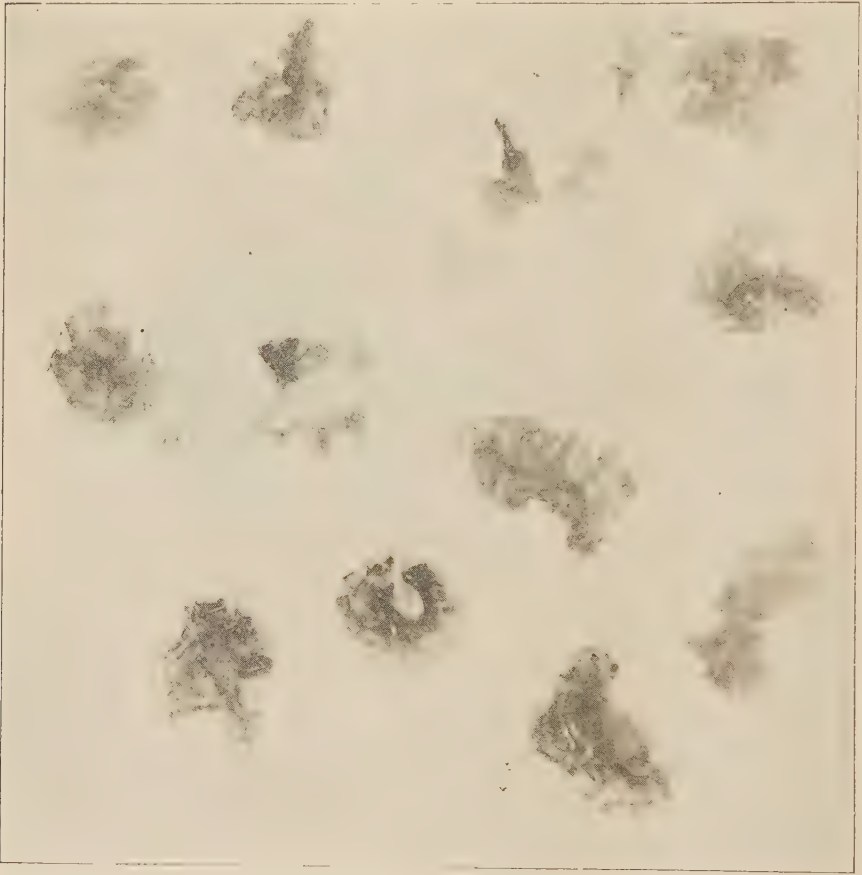


Fig. 10. Prothallia and young sporophytes from various nutrient solution cultures.

Solution IIId. No germination occurred in any of the cultures.

Solution IIe. No germination in any of the cultures.

Solution IIIf. No germination.

Solution IIg. No germination.

Solution III (Sachs' solution, unmodified). The prothallia were regularly dioecious and most of them were females. They were heart-shaped,

notched, and averaged about 8×3 mm. in size. All seemed normal and in excellent condition. The male prothallia, which were few in number, resembled those of other cultures. Young sporophytes in all stages of development were present.

Solution IIIa. Growth and development were rapid and no monoecious prothallia were observed. More than two-thirds were female, and were very distinctly heart-shaped, notched and generally larger than the female prothallia in solution III. The prothallia appeared healthy, the cells were very turgid, and many young sporophytes were developed.

Solution IV (Prantl's solution, unmodified). Germination of spores and growth of prothallia were excellent. The first antheridia and archegonia were observed six weeks after the spores were sown. Seven weeks later more than two-thirds of the prothallia were females and ranged in size from 10×5 mm. to 5×4 mm. They were distinctly notched, with a well developed meristem bearing archegonia in various stages of development.



Fig. 11. Young sporophyte growing in Knop's solution.

Archegonia were not infrequently found upon the dorsal surface. *In these cultures, as in the soil cultures, new archegonia were formed long after fertilization had taken place.* The cellular structure of the prothallia and the development of the rhizoids were normal, though rhizoids were sometimes found upon the dorsal as well as upon the ventral surface. A few monoecious individuals occurred in older cultures, these being of the same size and general appearance as the females. All the male gametophytes were small here, and varied in shape, some being irregular and some elongated with slightly heart-shaped apices. Young sporophytes in various stages of development were observed; some of these lived three or four months upon the solutions and formed a well-developed root and several fronds.

Solution IVa. The time required for spore germination and the rate of

growth of the young gametophytes for the first two weeks, were here approximately the same as in the complete Prantl's solution (IV). From this time on growth was slower; by the end of the third week these prothallia were only one-half as large as those in the full solution. Three weeks later, at the same time as in the full solution cultures, antheridia and archegonia were observed. About one-third of the prothallia were females and two-thirds males. Greater uniformity in size and shape was here apparent, among both males and females, than in any of the other solution cultures. The average size of the prothallia was about 2×1.5 mm. The majority were heart-shaped, very slightly notched and appeared to be in perfectly healthy condition. Rhizoids were here much more numerous and much longer than in any other of the solution cultures. The female prothallia had a well-developed meristem on which the archegonia were borne. Occasionally an individual was found with one or two archegonia

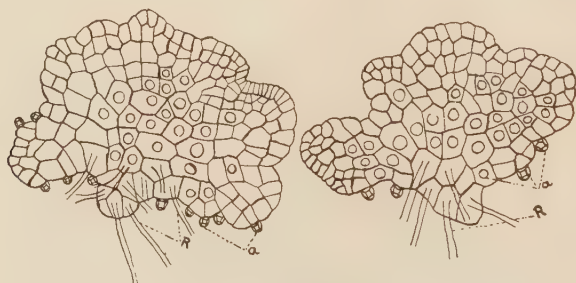


Fig. 12. Dioecious, male gametophytes from solution IVa, without nitrogen. a, antheridia; R, rhizoids.

on the dorsal surface. No monoecious prothallia were observed. That the reproductive organs were perfectly normal, was shown by the presence of many young sporophytes. (Figures 12 and 13.)

Solution IVb. Both germination of spores and growth and development of prothallia and reproductive organs were perfectly normal. The cellular structure appeared good. The majority of the prothallia were female, of the characteristic shape, and varied in size from 7×4 mm. to 4×1.5 mm. Male prothallia of the two types, regular and irregular, were present in larger numbers than in the complete Prantl's solution (IV). A few monoecious individuals were present. Sporophytes in various stages of development were also present.

Solution IVc. Germination and development were the same as in the full Prantl's solution (IV). Although the majority of individuals were dioecious, more monoecious ones were observed in these cultures than in those of the full solution. The female prothallia ranged in size from 7

$\times 3$ mm. to 3×1.5 mm. The monoecious prothallia resembled the females in size and shape, but some of them exhibited structural peculiarities. Antheridia frequently developed on the meristem, among the archegonia, and one case was observed in which they developed from the neck cells of archegonia. Several archegonia were observed with single egg cells and two distinct necks. The male prothallia showed no unusual form or structure.

Solution IVd. Spore germination was very good. The growth of prothallia was not rapid and the cultures, as a whole, did not appear to be healthy. The reproductive organs appeared at the usual time, the majority of the prothallia being females and varying in size from 6×3 mm. to 4×2 mm. The male prothallia were of the two forms above mentioned. A few monoecious individuals were observed. Young sporophytes were also present.

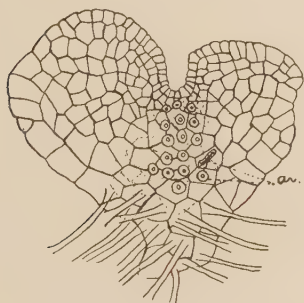


Fig. 13. Dioecious, female gametophyte from solution culture as in Fig. 12. *ar*, archegonia.

and shape among the prothallia was not here as great. The females were a trifle larger and more distinctly notched than those in solution IVa, the average size being 3×1.5 mm. The males varied in size and shape and their structure seemed normal, although they appeared distinctly yellowish. Fewer and shorter rhizoids were developed than in solution IVa. No monoecious prothallia were here observed. Young sporophytes were present.

Solution IVg. The majority of these prothallia were female and averaged in size from 2×1.5 mm. to 3×1.5 mm., the largest measuring only 5×2.5 mm. The males were of the usual size and shapes. A few monoecious individuals were observed. All of the prothallia appeared normal and in healthy condition. Many young sporophytes were present.

Solution IVh. These prothallia showed the best growth and development found in the series. Almost all were female, the largest being 10×3 mm., while the average size was 8×3 mm. Most of the male pro-

Solution IVe. Most of the plants were dioecious, a few monoecious ones were observed. The monoecious and female individuals varied in size from 8×3 mm. to 5×2 mm. and all were in healthy condition. Both types of male prothallia were present. Numerous young sporophytes developed.

Solution IVf. Spore germination and later growth and development were approximately the same as in solution IVa. The proportion of male to female individuals was also the same as that in solution IVa, but the uniformity in size

thallia were of the regular type. No monoecious ones were observed. Many young sporophytes were present.

Solution IVi. Growth was fair, with a larger percentage of females than males. The average size of the females was from 4×2.5 mm. to 5×2.5 mm. No monoecious prothallia were observed. Young sporophytes were present.

For each of the following rubidium solutions besides the cultures kept in the bright light, an additional series was subjected to slightly reduced light. The apparatus used for obtaining different degrees of shading consisted of two ground glass boxes made by joining together the ends of four pieces of ground glass with gummed cloth strips ("passe partout"), subsequently well coated with asphalt varnish. This glass framework stood upon a ground glass plate and was covered with another similar plate. When it was desired to reduce the light still more these glass chambers were covered with white cheese-cloth fastened at the edges by gummed cloth strips.

Solution V (Rubidium solution, unmodified). One week after the spores were sown both sets of cultures showed slight indications of germination. Seven days later, germination had advanced somewhat in the full light cultures, the young prothallia being usually one to two cells in length. In the reduced light cultures there were no further indications of germination. At the end of two weeks two-thirds of the germinating spores in the full light were dead, while the remainder, which had reached a two or three celled stage, were unhealthy and soon died. All spores in the reduced light cultures were dead at this time.

Solution Va. Seven days after sowing a splitting of the outer spore case indicated the commencement of germination. In the cultures in reduced light the indications were more pronounced than in the others. One week later both series of cultures showed single celled prothallia each with a well developed rhizoid. By the end of the third week the individuals of all cultures had five or six cells, but they never developed further and soon died.

Solution Vb. Most of the spores in both series of cultures showed a splitting of the outer wall, but germination did not go further in the cultures in reduced light. In full light a few spores germinated, but these died in about three weeks, after the formation of two or three cells.

Solution Vc. Neither in full light nor in reduced light did germination ever advance beyond a splitting of the outer spore case.

Solution Vd. No germination occurred.

Solution Ve. Fourteen days after the spores were sown one-third of them, in both light intensities, had developed one cell with a rhizoid. Those

in the full light never developed further, while those in reduced light lived a week longer, producing two or three cells before death ensued.

Solution Vf. In the full light cultures germination had just begun one week after the spores were sown, while in the cultures in reduced light there were then already indications of the formation of rhizoids. One week later, only about one-third of the germinating spores in full light had begun to form rhizoids. In the reduced light the majority of the young prothallia had by that time produced from one to three cells. At the end of three weeks, all of the germinating spores in full light were dead, while those in reduced light had developed six or seven cells, but they died soon after.

3. Induced Monoecism.

Monoecious prothallia were produced by transferring male and female prothallia from the soil cultures to nutrient solutions, and from one solution to another. The prothallia were selected from the soil cultures under a reading glass supported by a clamp on a ring stand, a dissecting microscope proving unsatisfactory because of the limited field of vision and the difficulty of focusing upon the soil cultures. The reading glass gave a larger field and furnished a good working distance between it and the surface of the culture. The prothallia were removed from the soil with a sharp-pointed scalpel and placed in a drop of water on a slide. The soil particles were first removed under a dissecting microscope, and the objects were then carefully examined with low power of a compound microscope. As the prothallia would not float upon the solutions, it was necessary to supply some supporting medium. For this purpose cotton was first used but its liability to decay in the solutions rendered it unsatisfactory; also, the growing rhizoids became entangled among the cotton fibers and were apt to be injured when the prothallia were removed for examination or transfer to fresh solutions. Glass wool was tried and likewise proved unsatisfactory; aside from unpleasantness in handling, the rhizoids were injured as they grew about in the wool. Glass beads of two sizes were used very successfully as a support for the larger female prothallia, but could not be used for supporting the smaller males. Small beads, 2 mm. in diameter, proved best. An advantage in the use of such beads was the ease with which they could be cleaned and sterilized, a disadvantage was the difficulty in packing them close enough together so that they could not roll slightly when the cultures were moved, thus allowing the prothallia to slip down between them. Sand proved the most satisfactory supporting medium in many ways, especially for the small male gametophytes. Perfectly clean, pure, white sand was placed in small glass capsules

and saturated with the nutrient solution, none of the solution being allowed to stand above the sand surface.

Transfer of female gametophytes from soil to solution.

The female prothallia transferred from the soil to the nutrient solutions were approximately normal and healthy. They were large, heart-shaped, distinctly notched, with well developed meristem bearing archegonia in different stages of development.

The following nutrient solutions, prepared according to the formulas already given (see page 99), were here employed: I (Beijerinck's solution), Ia, Ic; II (Knop's solution), IIc IIId, IIe IIIf, IIg; Vb (Rubidium solution, modified) and Ve. With each of these solutions three series of cultures were prepared, series 1 kept in bright light, series 2 in slightly reduced light (in the ground glass box) and series 3 in reduced light (in the glass box covered with white cloth). The results from these cultures were as follows, after from four to twenty-eight days.

Solution I (Beijerinck's solution, unmodified). *Series 1.* The majority of the prothallia became monoecious in from four to seven days after the transfer. Antheridia were produced both from marginal cells and from ventral surface cells on the wings, near the apex. Death immediately followed in the first prothallia transferred. This seemed to be caused, in some cases by too long exposure to bright sunlight, in others by injury brought about in transferring. Later, by improved technique, the latter difficulty was overcome.

Series 2 and 3. The majority of the individuals became monoecious after from fourteen to twenty-eight days, antheridia being produced in large numbers, both from the marginal cells and those of the ventral surface. Some prothallia gave rise to numerous proliferations, either in the form of filaments bearing antheridia at their apices, or in thallus form, the latter being distinctly notched and bearing antheridia from the marginal and ventral surface cells.

Solution Ia. No results.

Solution Ic. Series 1. No results.

Series 2 and 3. Practically the same results were obtained here as in series 2 and 3 with the complete solution (I).

Solution II (Knop's solution, unmodified). *Series 1 and 2.* No results were obtained. About one-half of the individuals transferred in each series lived and appeared to be in a normal healthy condition.

Series 3. Seventy-six per cent. of the prothallia transferred lived and fourteen per cent. became monoecious. All but one were in a healthy condition.

Solution IIc. Series 1. Sixty per cent. of the prothallia transferred lived and forty per cent. became monoecious. These were in a fair condition.

Series 2. Fifty-five per cent. of the prothallia transferred lived and nine per cent. became monoecious. These prothallia appeared healthier than those in series 1. New archegonia were formed.

Series 3. No results. Approximately one-half of the individuals died.

Solution IId. No results. All the prothallia lived and the majority appeared in good condition.

Solution IIe. Series 1. All the prothallia lived, ten per cent. became monoecious. The monoecious prothallia did not appear as healthy as the female prothallia.

Series 2 and 3. No results. All the prothallia lived and were in a healthy condition.

Solution IIf. Series 1 and 2. No results. All the prothallia lived and were in good condition.

Series 3. All the prothallia lived and twenty per cent. became monoecious. These were in poor condition.

Solution V (Rubidium solution, modified). Series 1 and 2. No results.

Series 3. Ninety-eight per cent. of the prothallia transferred lived and thirty-one per cent. became monoecious. All were unhealthy.

Solution Ve. Series 1. Fifty-five per cent. of the prothallia transferred lived and nine per cent. became monoecious. The monoecious prothallia did not appear to be as healthy as the females.

Series 2. Fifty-one per cent. of the prothallia transferred lived and six per cent. became monoecious. None of the prothallia appeared to be healthy.

Series 3. Sixty-nine per cent. of the prothallia transferred lived and eleven per cent. became monoecious. All were in poor condition.

Effect of chloroform. With the aim of determining whether chloroform might induce monoecism, four sets of cultures, each of ten female prothallia, were made with solutions I and Ve, and placed in an atmosphere containing chloroform. These cultures, each with a control without chloroform, were exposed to the following light conditions: bright light, slightly reduced light (in the glass box), reduced light (in the glass box covered with white cloth) and darkness. Three days later only two monoecious prothallia were found in the cultures in the atmosphere of chloroform and two in those of the controls. From this it appears that chloroform is not effective to produce monoecism, at least in the concentration and with the other conditions here employed.

Transfer of male gametophytes from soil to solution.

The male prothallia transferred from the soil to the nutrient solutions were of the two kinds previously described; one being irregularly shaped,

with one layer of cells and bearing antheridia on both surfaces and on the margins, while the other was more regular, somewhat elongated, with a slightly heart-shaped apex, also bearing antheridia as above. The second

type appeared much more vigorous and healthy than the irregularly shaped ones. All the individuals were approximately ten to twelve weeks of age when transferred. The same methods of selection and transfer were employed as for the transferred female prothallia; the only supporting medium that proved satisfactory was sand. Over one hundred prothallia were transferred to Beijerinck's and Knop's solutions; one series of cultures was placed in bright light, another series in slightly reduced light, and a third series in reduced light. All of the small, irregularly shaped prothallia died. Four of the regularly shaped individuals lived, and these became monoecious in four to six weeks after they had been transferred. Three of these grew somewhat in length, and the apices broadened and developed into distinctly heart-shaped, notched prothallia with meristems bearing archegonia on the ventral surfaces. (Figure 14.) The fourth prothallium, which was originally more heart-shaped and less elongated than the other three, became monoecious by the development of a meristem upon which were borne the archegonia. (Figure 15.)

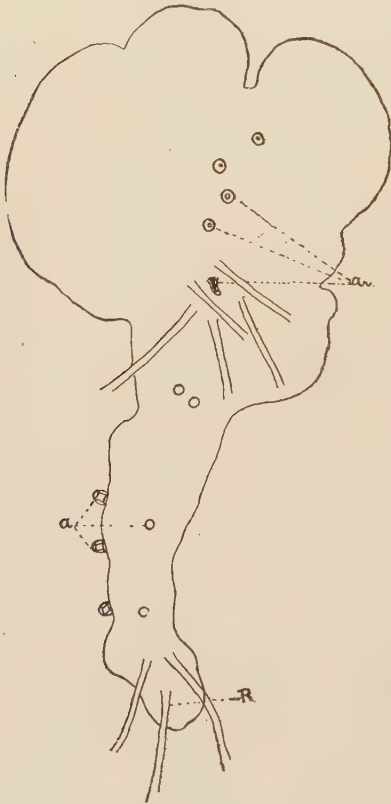


Fig. 14. Male gametophyte transferred from soil culture to solution and supported by pure sand, showing new growth (enlarged end) bearing archegonia. *a*, antheridia; *ar*, archegonia; *R*, rhizoids.

Transfers from one solution to another.

A further attempt to produce monoecism was made by transferring female and male gametophytes from one nutrient solution to another, the cultures standing in bright light. The characterization of the transfers and the resulting responses are presented below.

Transfer of females.

1a. From solution IV to solution I. No results. Growth greatly stimulated.

1b. From solution IV to solution Ic. No results. Growth as above.

1c. From solution IVa to solution I. No results. Growth as above.



Fig. 15. Portion of male gametophyte transferred from soil to Knop's solution and supported by pure sand, showing advent of monoecism by production of archegonia, also showing antheridia.

2a. From solution II to solution Ib. Monoecious prothallia were obtained.

3a. From solution VI to solution Ia. No results.

3b. From solution VI to solution II. No results.

4a. From solution III to solution II. Monoecious prothallia were obtained; adventitious shoots and young sporophytes were developed.

4b. From solution III to solution I. No results.

4c. From solution III to solution Ic. No results.

- 5a. *From solution IIIa to solution I.* No results.
5b. *From solution III to solution Ic.* No results.

Transfer of males.

The male prothallia transferred from one nutrient solution to another, were regular in shape, either elongated or heart-shaped, slightly notched, one layer of cells in thickness, and bore antheridia on the surfaces and margins. The following transfers were made.

1. *From solution IVc to solution IV.*
2. *From solution IVg to solution IVc.*
3. *From solution IVi to solution IVe.*

Four weeks after the transfer the prothallia in all the cultures became monoecious, by the development of a meristem bearing archegonia. All had grown, and varied in size from 4×3 mm. to 5×3 mm. While distinctly heart-shaped, most of them were only slightly notched.

GENERAL DISCUSSION.

The discussion of the different phases of this investigation may be presented under three headings: (A) Vegetative growth and development, (B) Sexual reproduction and (C) Sex latency.

A. VEGETATIVE GROWTH AND DEVELOPMENT.

As previously stated, the study of the germination of the spores was emphasized only for the purpose of determining the most favorable physiological conditions for rapid germination. The time of germination was found to be the same in the soil and in the nutrient solution cultures, with the exception of solutions Ve and Vf, of the rubidium series. Here, the higher concentrations of rubidium hydrate seemed to exert the same inhibitory influence upon germination as was exerted by the lower concentrations upon the later growth and development of the prothallia. The best germination was obtained in all the cultures by exposing them to bright sunlight.

The rate of growth was approximately the same in both soil and solution cultures during the early stages of development. Later, growth varied somewhat, especially in the nutrient solutions. Mature prothallia in the solutions favorable to growth were, on an average, larger than those in the soil cultures.

Most of the nutrient solutions used seemed to favor good growth, but detailed examination of the results given above shows certain prominent differences in form, growth, and development between the different cultures. This is in keeping with the results of other investigators, for fern

prothallia as well as for many other lower plants. Klebs' [93] work clearly demonstrated this. Osterhout,¹⁸ in his experimental work on balanced solutions, found various differences in the form and growth of thallus and rhizoids in the liverworts and in *Equisetum*, depending on the nutrient medium. Livingston¹⁹ observed two very distinct and easily distinguishable forms of *Stigeoclonium* according to the culture medium in which the alga was grown.

Several important facts were brought out by the growth and development of the gametophytes in solutions IVa and IVf (of the Prantl series). In solution IVf ammonium nitrate was omitted and the amount of sodium chloride was increased from 0.23g. to 2.3g., in the formula given on page 100.

The first of these facts demonstrates the extremely small amount of nitrogen which is necessary for the full development of prothallia and reproductive organs in this plant. Nitrogen is known to be one of the elements required by plants, and, since it has not yet been demonstrated that fern prothallia have the power of utilizing free nitrogen, it appears that the very small amount of this element present in the spore and spore case must have been sufficient, in these experiments, for the needs of the prothallium throughout its life history. Another fact shown by these cultures was a great increase in the number and length of rhizoids. A further discussion of this point will be taken up in a later paragraph. A third point of importance was the accelerating influence exerted upon growth by an increased concentration of sodium chloride in the absence of ammonium nitrate. This is especially interesting as Osterhout [09] has demonstrated that an antagonism exists between sodium and ammonium.

Sodium, as was to be expected, did not seem to be necessary for either growth or the development of reproductive organs. In fact, the very best growth was obtained in solutions in which this element was not included. When present it gave more favorable results as phosphate than as chloride. The early experiments of Birner and Lucanus²⁰ showed that sodium sulphate favored the entrance of phosphoric acid into the plant; while it lowered the rate at which calcium was taken up. By the addition of sodium chloride to the nutrient solution, calcium, sulphur and phosphorus were absorbed more slowly, but the percentage of magnesium and potassium in the plant was increased. Benecke²¹ suggests that sodium takes the place of potassium in osmosis, thus helping to sustain turgor in the plant. Copeland²² also ascribes to sodium the power of acting as either a direct

¹⁸ Osterhout, W.J.V., The nature of balanced solutions. *Bot. Gaz.* 47: 148. 1900.

¹⁹ Livingston, B. E., On the nature of the stimulus which causes the change of form in polymorphic green algae. *Bot. Gaz.* 30: 289. 1900.

²⁰ Birner, H., and Lucanus, B., Wasserkulturversuche mit Hafer in der Agric.-chem. Versuchstation zu Regenwalde. *Landw. Versuchsst.* 8: 140. 1866. (Quoted from Wheeler and Hartwell [06].)

²¹ Benecke, W., Untersuchungen über den Bedarf der Bakterien an Mineralstoffen. *Bot. Zeitg.* 65: 1. 1907.

²² Copeland, E.B., The relation of nutrient salts to turgor. *Bot. Gaz.* 24: 399. 1897.

or an indirect factor in sustaining turgor. He observed poor growth but high turgor when phosphorus, magnesium, and sulphur were omitted; while in the absence of potassium, growth was stunted and turgor decreased. Hartwell, Wheeler and Pember's experiments²³ with seedlings of millet, barley and rye indicated that the addition of sodium gave no increase in growth when potassium was abundant, but when potassium was deficient in the culture the addition of sodium proved beneficial. Schimper²⁴ considered the salts of potassium and oxalic acid, in the absence of calcium, to be poisonous. Based upon this consideration, Hartwell, Wheeler and Pember [07] suggest that sodium may possibly unite with oxalic acid as the latter is carried from one part of the plant to another, the acid being subsequently precipitated as the calcium salt. Osterhout [09], in a series of experiments on algae, liverworts, *Equisetum* and some thirteen genera of the flowering plants, demonstrated that both sodium and potassium could exert toxic effects; while Livingston [00] observed that sodium was a little more poisonous than potassium for certain green algae. Kauffman²⁵ in his work on the *Saprolegniaceae*, found that sodium and ammonium phosphate exerted, as they did in Klebs' experiments,²⁶ an inhibitory action upon the formation of reproductive organs in *Saprolegnia mixta*; no antheridia or oogonia were formed. Kauffman also observed that, when sodium chloride was added to a haemoglobin solution along with potassium phosphate, it exerted a stimulating effect upon reproduction, which was shown by a decided improvement in the appearance of the oogonia and by an increase in the number of antheridia.

The nitrates, either of potassium, ammonium, or calcium, when increased in concentration up to a certain limit, tended to stimulate growth in these fern gametophytes; above this limit, however, these salts exerted an inhibitory effect. This was especially true of ammonium nitrate. When the concentration of ammonium nitrate in solution IV (Prantl's) was increased from 0.64g. to 6.4g. per 1000 cc. of solution, the prothallia never reached maturity; growth was very slow, and the form was greatly distorted. The best growth was obtained with potassium or calcium nitrate. In solutions where the amount of potassium nitrate was increased, growth was very rapid and more nearly uniform for all individuals, and turgor was especially pronounced. The effect produced by the omission of nitrate from the solution has already been discussed. It was also observed that

²³ Hartwell, B.L., Wheeler, H.J., and Pember, F.R., The effect of the addition of sodium to deficient amounts of potassium upon the growth of plants in both water and sand cultures. Rhode Island Agric. Exp. Sta. Report 20, part 2: 299. 1907.

²⁴ Schimper, A.F.W., Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora 73: 207. 1890.

²⁵ Kauffman, C.H., A contribution to the physiology of the *Saprolegniaceae*, with special reference to the variations of the sexual organs. Ann. Bot. 21: 361. 1908.

²⁶ Klebs, G., Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena. 1896.

if a nitrate was supplied, ammonium or calcium could be omitted without materially affecting either the rate or amount of growth. This seems to indicate, either that ammonium and calcium were not necessary for the development of the prothallia, or that the small amount of these substances present in the spore and spore case proved sufficient for the needs of the organism. The results obtained when calcium was omitted are especially interesting, as Reed²⁷ found that spores of *Gymnogramme sulphurea* germinated and produced normal prothallia in solutions without calcium salts. By substituting magnesium for the missing calcium in his solutions, Reed obtained good growth; antheridia were produced but no archegonia. Other experiments by the same writer indicated further, that a small amount of calcium sufficed for the nutrition of most plants and that this element was beneficial in its power as an antidote for magnesium. Schimper [90] and Groom [96] attributed the function of calcium to the neutralizing of oxalic acid, while Kraus [97], Loew [02], Bruch [02], and Grafe and von Portheim [06] opposed this view. Benecke [07] found, in experiments with *Spirogyra*, that an antagonism existed between magnesium and calcium. Loew [99] and Kearney and Cameron [02] agreed with this view. Molisch [96] found calcium necessary for wall formation in *Spirogyra*. Kauffman's cultures of *Saprolegnia* in calcium nitrate and calcium phosphate solutions showed several cross walls separating the hypogynous cell from the oogonial stalk. The recent work of Osterhout has shown, without a doubt, that an antagonism exists not only between sodium and calcium, but also between magnesium and calcium.

Another point that is worthy of consideration is the effect of rubidium hydroxide as shown in the present studies. This substance was used in connection with Knop's solution (IIa) and also in a special series of solutions in which the amount of rubidium hydrate, as well as of the other compounds, was varied (Va. Vf). Germination failed in the latter series, so conclusions must be based upon the results obtained with the former. No difference could be noted between cultures in unmodified Knop's solution and those with addition of rubidium hydrate, either in the germination of the spores or the early growth of the gametophytes. Later, growth became slower, however, and the fully developed prothallia were smaller in the presence of the rubidium compound. Monoecism was greatly increased by rubidium hydrate, as shown by the fact that *sixty per cent. of the prothallia* in solution IIa were monoecious. It appears that the rubidium compound here exerted a retarding effect upon growth and that the vitality of the prothallia was thus sufficiently lowered to stimulate a latent male tendency to activity and yet not kill the organisms. There has been much discussion regarding the effect of rubidium salts upon

²⁷ Reed, H., Value of certain nutritive elements to plant cells. *Ann. Bot.* 21: 501. 1907.

plants and the ability of these salts to replace potassium in plant nutrition. Birner and Lucanus [66], with water cultures of oat plants, found potassium to be absolutely indispensable for growth and development, and that it could not be replaced by rubidium, caesium, sodium, lithium or ammonium. These results were confirmed by the observations of Hellriegel [67]. Benecke [07] found rubidium capable of replacing potassium in the nutrition of *Bacillus fluorescens* and *B. pyocyaneus*. Molisch's [96] work showed that for *Protococcus infusionum* the chemically related elements rubidium, caesium, lithium and sodium could not be substituted for potassium. Loew,²⁸ working with lower plants, demonstrated that rubidium could replace potassium. He also found that when all necessary elements, including potassium, were present rubidium exerted an accelerating effect upon growth. Kauffinan's [08] cultures of *Saprolegnia* in haemoglobin solutions to which rubidium hydroxide had been added, showed good vegetative growth but the development of sexual organs was here inhibited. All this evidence seems to indicate that the effect of rubidium is not the same in all cases, but varies with the nature of the plant.

Since the early work of Leitgeb [77] and of Prantl²⁹, which demonstrated the dorsiventrality of fern prothallia, the lower surface, as the shaded side, has been considered the normal region for the development of rhizoids and reproductive organs. The majority of the rhizoids in my experiments, however, developed from the upper surfaces of the prothallia although these surfaces were fully exposed to bright light. This was especially true in the solution cultures, where water was always present on the lids of the capsules. It was also true in the soil cultures which were kept so moist that drops of water collected on the glass plates covering the pots. Light conditions were suggested by Peirce [06] as controlling the origin of rhizoids upon the various sides of prothallia of *Gymnogramme triangularis*, but such a supposition will not prove satisfactory here; the gametophytes of my experiments did not receive equal illumination on the two sides. The moisture conditions were more nearly alike on the two sides, however, and it may be that moisture plays an important part in this control, though light cannot be left out of consideration. That moisture may play an important part in the development of rhizoids was demonstrated by Dachnowski³⁰ in his experiments with *Marchantia polymorpha*.

²⁸ Loew, O., Zur Frage der Vertretbarkeit von Kaliumsalzen bei niederen Pflanzen. Bot. Centralbl. 74: 202. 1808.

²⁹ Prantl, K., Ueber die Anordnung der Zellen in flächenförmigen Prothallien der Farne. Flora 36: 407, 529, 545. 1878.

³⁰ Dachnowski, A., Zur Kenntnis der Entwicklungs-Physiologie von *Marchantia polymorpha*. Jahrb. wiss. Bot. 44: 254. 1907.

The number and size of the rhizoids of the gametophytes here dealt with seem to be closely related to the nutrient conditions. Fewer rhizoids were generally developed in the solution cultures than in those with soil. In solutions that were favorable to good growth, rhizoids were fewer in number, shorter and broader; while in solutions which did not favor growth, they were more numerous, longer, more slender and darker in color. Benecke [07] observed that the development of the rhizoids of *Lunularia* was influenced by the absorption of chemicals from the glass of the vessels in which they were cultivated. A physiological analogy would seem to exist between the development of rhizoids and the development of root hairs in higher plants. That the development of root hairs is related to the amount of water present is well known; when the roots of seedlings are grown immersed in water no root hairs or a reduced number develop. Here the cells of the root are in direct contact with water and can directly absorb it. In prothallia growing on the surface of a solution the cells are also in contact with water and direct absorption must be possible. When all the elements necessary for growth are present in sufficient quantities to meet the needs of the prothallia the stimulus for rhizoid formation appears to be weakened. If the necessary elements are not present, or the quantity is insufficient for growth, this stimulus seems to be intensified.

Adventitious outgrowths from the gametophytes were frequently developed in all the cultures. In some cases these outgrowths were mere filaments of a few cells and bore antheridia near their tips. In other cases they developed into slightly elongated prothallia with heart-shaped, notched apices and well-developed meristems bearing archegonia. Similar outgrowths have been described for other ferns by Wiegand [49], Hofmeister [51], Kny [70], Goebel [77], DeBary [78], Bauke [78], Dodel-Port [80], Klebs [93], Heim [96], Lagerberg [06], Pace [10], Heilbronn [10], and Fischer [11]. Klebs interprets the development of these outgrowths as being due to weak light. While weak light seems undoubtedly to be the cause of their origin in some cases, it cannot be considered the determining factor in all cases in the present work, for such outgrowths developed in bright light from old prothallia, or prothallia which had been transferred either from soil to solution or from one solution to another. A comparison of these results with those of previously mentioned workers indicates that, whatever factor may have been the determining one, their development was intimately connected with the vitality of the prothallia, outgrowths usually appeared when vitality was lowered, either through old age or through a shock due to unfavorable conditions.

B. SEXUAL REPRODUCTION.

The gametophyte of *Onoclea Struthiopteris* exhibits several phases of development which seem to depend largely upon environmental factors. Under conditions of poor nutrition male prothallia were developed in these studies, while under conditions of good nutrition female prothallia were in evidence. When male individuals were subjected to *favorable* nutrition there occurred a progressive development in the prothallia, shown by growth and the formation of a meristem with archegonia. If, on the other hand, female prothallia were subjected to *unfavorable* nutrition a retrogression resulted, shown by a decline in vitality and the development of antheridia. This suggests that the male tendency is associated with a lower type, and the female tendency with a higher type of development in the gametophyte. That male prothallia were developed in response to poor nutrition was shown by the solution cultures. In solutions which furnished the best cultural conditions fewer male individuals were developed and the majority of these were regular in shape. In solution cultures not as well suited to growth the proportion of irregularly-shaped males was much greater. The association of "maleness" and "femaleness" with a difference in the size and shape of the prothallia, due to unequal development, has been observed for the gametophytes of other ferns. Attention has been especially called to the "weak" development of the male gametophyte by Schacht [49], for *Pteris serrulata*; by Cornu [74], for *Aspidium filix-mas*; by Prantl [81], for *Osmunda* and some other ferns; by Atkinson [94], for *Niphobolus crispum corymbiferum*; by Heim [96], for *Lygodium japonicum*, *Balantium antarcticum*, *Alsophila australis*, and *Doodyacaudata*; by Life [06], for *Dicksonia apiifolia*, *Aneimia phyllitidis*, *Alsophila pruinata*; and by Lagerberg [06], for *Pteridium aquilinum*.

That the unequal development of the gametophytes is more dependent upon the factors of environment than upon inheritance is shown by the fact that most of the dioecious prothallia, when placed under different conditions of nutrition, developed the other sex. The experimental evidence here presented has clearly shown that this is true of *Onoclea struthiopteris*; not only the female prothallia, but also the males, were induced to become monoecious when placed under proper environmental conditions. Frequent reference occurs in the literature to similar observations made upon various other gametophytes. Bauke³¹ speaks of an induced monoecism in *Aneimia phyllitidis*, stating that female prothallia over which moss protonema had grown developed antheridia. Also, female prothallia in cultures of *Lygodium japonicum* formed antheridia through the influence of unfavorable conditions. Prantl [81] caused "ameristic" prothallia to

³¹ Bauke, H., Zur Kenntnis der sexuellen Generation bei den Gattungen *Platyserium*, *Lygodium* und *Gymnogramme*. Bot. Zeitg. 36: 753. 1878.

become monoecious by transferring them from a nitrogen-free solution to one containing nitrogen, where they developed a meristem and archegonia. Buchtien³² conducted experiments on the gametophyte of *Equisetum pratense* and showed that when female prothallia were transferred from fertile sand to sterile sand they developed antheridia and became monoecious. Peirce [06] found that it was possible completely to suppress the development of the reproductive organs of *Gymnogramme triangularis* by insufficient light. Klebs [93] not only showed that it was possible by the reduction of light intensity to suppress the reproductive organs and keep the prothallia of *Pteris aquilina* in a vegetative stage, but that antheridia were formed when these prothallia were placed in weak light. If these prothallia were placed in stronger light archegonia were developed.

Campbell³³ states that as soon as one archegonium of a gametophyte of this fern is fertilized, new ones cease to be formed. This was not the case in either my soil or solution cultures, for prothallia were observed which produced new archegonia even after fertilization had taken place and both antheridia and archegonia were produced in some cases after the young sporophyte was well developed. As in the case of the rhizoids, the shaded surface has been considered the normal one for the development of the reproductive organs. Archegonia frequently developed, however, upon the upper surfaces of female prothallia in both the soil and solution cultures. The occurrence of these organs on the upper surface can not be explained as due to the influence of light, for in the majority of cases this side was exposed to full insolation. Similar cases have been reported by Peirce [06] and others. Peirce's prothallia were grown in Knop's solution, on a clinostat and with approximately equal illumination on all sides. Here, as in the case of rhizoids, weak illumination fails to account for the development of archegonia on the upper surfaces in my cultures, for these surfaces received more light than did the under one.

A number of peculiarities were observed in the development of both antheridia and archegonia. In several cases archegonial neck cells gave rise to antheridia which appeared normal and healthy. The development of antheridia from neck cells of archegonia has been reported and figured by Miss Lyon³⁴ as occurring on a fern prothallium of undetermined species, probably an *Osmunda*. This had been reported earlier by Goebel³⁵ for *Hemomitis palmata* and *Lygodium japonicum*. The cases cited by Treub³⁶ and Hy³⁷ might also be considered as analogous. Treub observed an arche-

³²Buchtien, B., Entwicklungsgeschichte des Prothalliums von *Equisetum*. Dissertation. Rostock 1878.

³³Campbell, D.H., The structure and development of mosses and ferns. New York. 1905.

³⁴Lyon, Florence M., The evolution of sex organs of plants. Bot. Gaz. 37: 280. 1904.

³⁵Goebel, K., Organographie der Pflanzen. Jena. 1808.

³⁶Traub, M., Recherches sur les Lycopodiacees. Ann. Jard. Bot. Buitenzorg 3. 5, 7, 8. 1884-1890.

³⁷Hy, F., Recherches sur l'archegone et le développement du fruit des Muscinees. Ann. Sci. Nat. Bot. VI. 18: 21. 1884.

gonium of *Lycopodium phlegmaria* in which the upper canal cells divided to form a large number of cells which became spermatogenous in character. Hy observed a similar case in the mosses. Goebel [01] interpreted this general phenomenon as the result of old age. Such an explanation proves unsatisfactory for the cases observed by me, as these prothallia, like the one cited by Miss Lyon, were surely in a healthy condition. In certain other cases observed, the archegonia had developed two complete necks with but a single egg cell.

Antheridia developed on the upper as well as on the lower surfaces of the gametophytes here studied and were not restricted to any particular region of these surfaces. In some of the monoecious individuals antheridia developed among the archegonia on the meristem. Another instance of this peculiarity was observed by Miss Pace [10]. A case was also observed where two antheridia had developed upon a single stalk. This was similar in appearance to the one reported and figured by Miss Lyon.

C. SEX LATENCY.

The question of sex latency in the gametophyte generation of *Onoclea struthiopteris* is an extremely interesting one, as the genus *Onoclea* has been considered as dioecious by most writers. Campbell [87], who worked out the morphological life history of this fern in detail, emphasized "the dioecism displayed by the prothallia." That dioecism cannot be considered a marked or fixed characteristic of the prothallia of *Onoclea* has been clearly shown by the following lines of evidence as set forth in this investigation.

(1) Monoecious prothallia were frequently found.

(2) Monoecious prothallia occurred in cultures with various nutrient solutions.

(3) Dioecious prothallia, both males and females, were induced to become monoecious when placed under certain culture conditions.

In addition to the above evidence are the recorded observations of bisexual prothallia by Lagerberg [06], and Mottier [10].

All this evidence indicates that the sex of the gametophyte generation of *Onoclea struthiopteris* is not fixed in the spores; but that tendencies toward the production of both sorts of organs are present in the cells of the gametophytes. Therefore, there appear to be no purely male nor purely female prothallia, and Mottier's terms "purely male or female gametophytes" are hardly applicable. Among the dioecious prothallia it may be supposed that some environmental factor or combination of factors inhibits one tendency, either the male or the female, and allows the other to be effective. Thus the organisms are either male or female, as the case may be, and the other tendency is latent. In the monoecious prothallia, the

influence of environmental factors or combinations of factors appears to be such that both sex tendencies are equally stimulated to become effective, and both kinds of reproductive organs are produced.

As the larger percentage of monoecious prothallia was found in the older cultures, it appears as though some physiological change took place in the cells during development, and it may be supposed that this change permits the previously latent tendency to be more easily stimulated to activity or makes this tendency less easily inhibited. The male tendency may be considered as being thus latent in all the cells of the female gametophytes; for any cell, including those which form the meristem and the archegonial necks, may produce an antheridium. This evidence supports the theory advanced by Miss Lyon [04] concerning the plasticity of the cells forming the reproductive organs. It also supports the theory, advanced by Miss Ferguson,³⁸ that it "might be that maleness and femaleness have no necessary relation to the form or position of sexual organs, but that the sperms and eggs are developed in relation to the sexual act or process, not as a necessary expression of sexuality."

The majority of the female prothallia which became monoecious appeared to be less healthy than those which remained female. Here it seems that monoecism was associated with lowered vitality. The latent male tendency had been stimulated to become active, through the influence of certain environmental factors, which, separately or together, also lowered the vitality of the prothallia. The failure of some female prothallia to become monoecious is not apparently due to absence of the latent male tendency, but to failure of the environment so to reduce the vitality of the cells as to call forth this tendency.

The fact that apparent differences in the vitality of the male gametophytes here studied were associated with variety of form, may indicate that the different forms of prothallia were developed in response to environmental conditions. The irregularly shaped male gametophytes, being in a poorly nourished condition, could not survive the shock of transfer from soil to solution, or from one solution to another. If this difficulty could have been overcome, so that these prothallia might have been placed under the best conditions of nutrition, there seems to be no obvious reason why they could not have developed into normal, healthy, monoecious prothallia, in the same manner as did the more or less regularly shaped males when the latter were transferred.

The effect of environmental factors upon sex differentiation in the ferns was first studied by Prantl [79]. In this work he seems to consider light as an important factor in sex control, for he had observed that strong light tended to produce only female prothallia, and weak light only male

³⁸ Ferguson, Margaret C., Imbedded sexual cells in the Polypodiaceae. Bot. Gaz. 51: 443. 1911

prothallia. In later work [81], the same author placed greater emphasis upon nutritive conditions, basing this opinion upon experimental evidence obtained by cultivating gametophytes on nutrient solutions. As he obtained only males on solutions from which nitrate was absent, he drew the general conclusion that an insufficient nitrogen supply might account for thickly grouped prothallia bearing only antheridia. This explanation fails, however, to account for the production of dioecious prothallia in *Onoclea struthiopteris*. In cultures made by sowing the spores upon Prantl's nutrient solution from which ammonium nitrate had been omitted, female prothallia were obtained. These were small, heart-shaped, notched prothallia with a well developed meristem bearing archegonia. The production of young sporophytes showed that these archegonia were normal and healthy. Furthermore, as has been previously stated, the position of the gametophytes in the soil cultures did not seem greatly to influence their development or sex; many of the smaller, "ameristic" prothallia, bearing only antheridia, were found in the less crowded regions of the cultures, while the larger, "meristic" ones, bearing only archegonia, were often found in more crowded regions. Monoecious prothallia were found in both regions.

Klebs' [93] work indicates that, for the particular ferns he worked with, light played a very important part in sex differentiation. Later work by Heim [96] and Peirce [06] points to the same conclusion. While we must admit that light may be an important factor, we must not lose sight of the possible importance of other factors of the environment. Even by cultivating prothallia upon nutrient solutions whose chemical constituents are known, we are far from having determined the effects of the chemical reactions which may result from the presence or absence of the different chemical elements. Our only hope of ever gaining such knowledge lies in further experimental study of solution cultures under known conditions of light and temperature.

Klebs has demonstrated that an intimate relation exists between growth and reproduction. Since growth is dependent upon nutrition, then nutrition is the most important factor both in growth and reproduction. It is a well known fact that growth can be carried on under conditions of nutrition which do not prove sufficient for reproduction. In the production of the egg cells and their necessary accessories, a greater demand is made upon the prothallia than results from the vegetative processes alone. In order to meet this demand greater growth must precede reproduction. The larger heart-shaped prothallia are able to meet this demand, having had a more vigorous growth, and hence they are able to develop female reproductive organs.

Viewed in the light of the plasticity of plant bodies in relation to their

environmental factors, it seems that sex is not predetermined in the spore of *Onoclea struthiopteris*, but that tendencies toward the production of both kinds of sex organs exist as potentialities in the cells of the prothallia, and are influenced by environmental factors to become active or to remain latent. These controlling factors are not only effective during the early development of the gametophyte but may continue so during the greater part of its life history.

LITERATURE CITED.

Numbers in brackets throughout the preceding pages refer to the year of publication and to the corresponding numbers which follow author's names in this list. Where more than one reference to the same year occurs under a given name these are serially numbered, in italics, within the brackets.

- Atkinson, G.F. [94], The study of the biology of ferns by the collodion method. New York. 1894.
- Bary, A. de [78], Ueber apogame Farne und die Erscheinung der Apogamie im Allgemeinen. Bot. Zeitg. 36: 449, 465, 481. 1878.
- Bauke, H. [78], Zur Kenntniss der sexuellen Generation bei den Gattungen *Platyserium*, *Lygodium* und *Gymnogramme*. Bot. Zeitg. 36: 753. 1878.
- Beck, G. [78], Entwicklungsgeschichte des Prothalliums von *Scolopendrium vulgare*. Bot. Zeitg. 36: 780. 1878. (Abstract.)
- Benecke, W. [07], Untersuchungen über den Bedarf der Bakterien an Mineralstoffen. Bot. Zeitg. 65 1. 1907.
- Buchtien, B. [87], Entwicklungsschichte des Prothalliums von *Equisetum*. Dissertation. Rostock. 1887.
- Birner, H., and Lucanus, B. [66], Wasserkulturversuche mit Hafer in der Agrik.-chem. Versuchstation zu Regenwalde. Landw. Versuchsst. 8: 128. 1866. (Quoted by Wheeler and Hartwell [08].)
- Black, C.A. [09], Development of the imbedded antheridia in *Dryopteris stipularis*. Bull. Torr. Bot. Club 36: 557. 1909.
- Borodin, J. [67], Ueber Farnprothallien. St. Petersburg. 1867.
- [68], Ueber die Wirkung des Lichtes auf einige höhere Kryptogamen. Bull. Acad. Sci. St. Petersburg 12: 432. 1868.
- Bower, F.O. [08], The origin of a land flora. London. 1908.
- Breazeale, J.F. [06], The relation of sodium to potassium in soil and solution cultures. Jour. Amer. Chem. Soc. 28: 1013. 1906.
- Bruch, P. [02], Zur physiologischen Bedeutung des Kaliums in der Pflanze. Landw. Jahrb. 30: 127. 1902.
- Burgerstein, A. [08], Einfluss des Lichtes verschiedener Brechbarkeit auf die Bildung von Farnprothallien. Ber. Deutsch. Bot. Ges. 26: 449. 1908.
- Cameron, F.K. [02]. See Kearney and Cameron [02].
- Campbell, D. H. [87], The development of the ostrich fern. Mem. Boston Soc. Nat. Hist. 4, No. 2. 1887.
- [91], A study of the apical growth of the prothallia of ferns. Bull. Torr. Bot. Club 18: 73. 1891.
- [08], Recent work upon the development of the Archegonium. Bot. Gaz. 26: 428. 1898. (Review.)
- [05], The structure and development of mosses and ferns. New York. 1905.
- Copeland, E.B. [97], The relation of nutrient salts to turgor. Bot. Gaz. 24: 399. 1897.
- Cornu, M. [74], Sur un semis de fougère (*Nephrodium felix-mas*). Bull. Soc. Bot. France 21. 1874.
- Dachnowski, A. [07], Zur Kenntnis der Entwicklungs-Physiologie von *Marchantia polymorpha* L. Jahrb. wiss. Bot. 44: 254. 1907.

- Dodel-Port, A. [85], Das amphibische Verhalten der Prothallien von Polypodiaceen. *Bot. Zeitg.* 38: 525. 1880. (Review.)
- Ferguson, Margaret C. [11], Imbedded sexual cells in the Polypodiaceae. *Bot. Gaz.* 51: 443. 1911.
- Fischer, Hugo [11, 1], Licht- und Dunkelkeimung bei Farnsporen. *Bot. Centralbl. Beih.* 27: 60. 1911.
- [11, 2], Wasserkulturen von Farnprothallien, mit Bemerkungen über die Bedingungen der Sporenkeimung. *Bot. Centralbl. Beih.* 27: 54. 1911.
- [12], Weiteres über Wasserkulturen von Farnprothallien. *Bot. Centralbl. Beih.* 28: 192. 1912.
- Goebel, K. [77], Entwicklungsgeschichte des Prothalliums von *Gymnogramme leptophylla*. *Bot. Zeitg.* 35: 671, 681, 697. 1877.
- [01], Organographie der Pflanzen. Jena. 1898.
- [87], Outlines of classification and special morphology. Translated by H.E.F. Garnsey. Oxford. 1887.
- Grafe, V., and Portheim, L. [06], Untersuchungen über die Rolle des Kalkes in der Pflanze. *Anz. K.K. Akad. Wiss. Wien Math-nat. hist. Klasse* 18: 324. 1906.
- Groom, P. [96], Preliminary note on the relation between calcium and the conduction of carbohydrates in plants. *Ann. Bot.* 10: 91. 1896.
- Hartwell, B.L. [07]. See Wheeler and Hartwell [07].
- Hartwell, B.L., Wheeler, H.J., and Pember, F.R. [07], The effect of the addition of sodium to deficient amounts of potassium upon the growth of plants in both water and sand cultures. *Rhode Island Agric. Exp. Sta. Report* 20, part 2: 299. 1907.
- Heald, F.D. [06], Toxic effect of dilute solutions of acids and salts on plants. *Bot. Gaz.* 22: 125. 1896.
- [08], Conditions for the germination of the spores of bryophytes and pteridophytes. *Bot. Gaz.* 26: 25. 1898.
- Heilbronn, A. [10], Apogamie, Bastardierung und Erblichkeitsverhältnisse bei einigen Farnen. *Flora* 101: 1. 1910.
- Heim, Carl [06], Untersuchungen über Farnprothallien. *Flora* 82: 328. 1896.
- Hofmeister, W. [62], Higher cryptogamia. Translated by F. Currey. London. 1862.
- Hy, F. [84], Recherches sur l'archegone et le développement du fruit des Muscinees. *Ann. Sci. Nat. Bot.* VI. 18: 21. 1884.
- Kahlenberg, L., and True, R.H. [96], Toxic action of dissolved salts. *Bot. Gaz.* 22: 81. 1896.
- Kauffman, C.H. [08], A contribution to the physiology of the Saprolegniaceae, with special reference to the variations of the sexual organs. *Ann. Bot.* 21: 361. 1908.
- Kaulfuss [27], Das Wesen der Farnkräuter. 1827. (Quoted by Heald [08].)
- Kearney, T.H., and Cameron, F.K. [02], Some mutual relations between alkali soils and vegetation. *U.S. Dept. Agric. Report* 71. 1902.
- Klebs, G. [93], Ueber den Einfluss des Lichtes auf die Fortpflanzung der Gewächse. *Biol. Centralbl.* 13: 641. 1893.
- [04], Ueber Probleme der Entwicklung. *Biol. Centralbl.* 24: 257. 1904.
- [96], Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena. 1896.
- Kny, L. [70], Ueber Bau und Entwicklung des Farnantheridiums. *Monatsber. K. Preuss. Akad. Wiss. Berlin.* 1870.
- Kraus, G. [97], Ueber das Verhalten des Kalkoxalats beim wachsenden Organe. *Flora* 83: 54. 1897.
- Laage, A. [07], Bedingungen der Keimung von Farn- und Moossporen. *Bot. Centralbl. Beih.* 21: 76. 1907.
- Lagerberg, T. [06], Zur Entwicklungsgeschichte des *Pteridium aquilinum*. *Arkiv. Bot.* 6, No. 5: 1. 1906.
- Leitgeb, H. [77], Ueber Bilateralität der Prothallien. *Flora* 60: 174. 1877.
- [79], Studien über Entwicklung der Farne. *Sitzungsber. K. K. Akad. Wiss. Wien, Math.-nat. hist. Klasse* 80, Abt. I. 1879.
- Life, A.C. [07], Effect of light upon the germination of spores and gametophytes of ferns. *Missouri Bot. Garden Report* 18: 109. 1907.
- Livingston, B.E. [00], On the nature of the stimulus which causes the change of form in polymorphic green algae. *Bot. Gaz.* 30: 289. 1900.
- [05], Chemical stimulation of a green alga. *Bull. Torr. Bot. Club* 32: 1. 1905.

- Livingston, E. B. [93], The rôle of diffusion and osmotic pressure in plants. Chicago. 1903.
- Loew, O. [91], Ueber die physiologischen Functionen der Phosphorsäure. Biol. Centralbl. 11: 273. 1891.
- [92], Ueber die physiologischen Functionen der Calcium- und Magnesiumsalze im Pflanzenorganismus. Flora 75: 368. 1892.
- [98], Ueber die physiologischen Functionen der Calciumsalze. Bot. Centralbl. 74: 257. 1898.
- [98], Zur Frage der Vertretbarkeit von Kaliumsalzen durch Rubidiumsalze bei niederen Pflanzen. Bot. Centralbl. 74: 202. 1898.
- [99], The physiological rôle of mineral nutrients. U.S. Dept. Agric. Div. Veg. Phys. and Path. Bull. 18. 1899.
- Lucanus, B. [66]. See Birner and Lucanus [66].
- Lyon, Florence M. [94], The evolution of sex organs of plants. Bot. Gaz. 37: 280. 1904.
- Milde, J. [53], Ueber eine eigenthümliche Form der fructificierenden Wedel von Struthiopteris germanica. Flora 11: 745. 1853.
- [64], Ueber Botrychien, deren Eintheilung und unterscheidung. Bot. Zeitg. 22: 101. 1864.
- Molisch, H. [95], Die Ernährung der Algen. Sitzungsber. K. K. Akad. Wiss. Wien, Math.-nat. hist. Klasse 104, Abt. I: 783. 1895.
- [96], Die Ernährung der Algen. Sitzungsber. K. K. Akad. Wiss. Wien, Math.-nat. hist. Klasse 104, Abt. I: 633. 1896.
- Mottier, David [97], The history and control of sex. Proceed. Indiana Acad. Sci. 28. 1907.
- [10], Note on the sex of the gametophyte of Onoclea struthiopteris. Bot. Gaz. 50: 209. 1910.
- Osterhout, W.J.V. [96], On the importance of physiologically balanced solutions for plants. I. Marine plants. Bot. Gaz. 42: 127. 1906.
- [97], On the importance of physiologically balanced solutions for plants. II. Fresh water and terrestrial plants. Bot. Gaz. 44: 259. 1907.
- [98, 1], The antagonistic action of magnesium and potassium. Bot. Gaz. 45: 117. 1908.
- [98, 2], Die Schutzwirkung des Natriums für Pflanzen. Jahrb. wiss. Bot. 46: 121. 1908.
- [99, 1], The nature of balanced solutions. Bot. Gaz. 47: 148. 1909.
- [99, 2], On similarity in the behavior of sodium and potassium. Bot. Gaz. 48: 98. 1909.
- Pace, Lula [10], Some peculiar fern prothallia. Bot. Gaz. 50: 49. 1910.
- Portheim, L. [96]. See Grafe and Portheim [96].
- Peirce, G.J. [96], Studies of irritability in plants. Ann. Bot. 20: 449. 1906.
- Pember, F.R. [97]. See Hartwell, Wheeler and Pember [97].
- Prantl, K. [78], Ueber die Anordnung der Zellen in flächenförmigen Prothallien der Farne. Flora 36: 497, 529, 545. 1878.
- [79, 1], Ueber den Einfluss des Lichtes auf die Bilateralität der Farnprothallien. Bot. Zeitg. 37: 713, 719. 1879.
- [79, 2], Ueber Anordnung der Zellen in Farnprothallien. Bot. Zeitg. 37: 789. 1879.
- [81], Beobachtungen über die Ernährung der Farnprothallien und die Vertheilung der Sexualorgane. Bot. Zeitg. 39: 753. 1881.
- Reed, H. [97], Value of certain nutritive elements to plant cells. Ann. Bot. 21: 501. 1907.
- [10], The effect of certain chemical agents upon the transpiration and growth of wheat seedlings. Bot. Gaz. 49: 81. 1910.
- Schacht, H. [49, 1], Beitrag zur Entwicklungsgeschichte des Sporangiums wie der Spore einiger Farnkräuter. Bot. Zeitg. 7: 547, 553. 1849.
- [49, 2], Beitrag zur Entwicklungsgeschichte der Farnkräuter. Linnaea. 22: 753. 1849.
- Schelting, A. [75], Einige die Entwicklung der Farnprothallien betreffende Fragen. Bull. K. Neuruss Univ. Odessa 17. 1875. Quoted by Laage [97]. [See also review, apparently of same publication: Bot. Jahresber. 3: 328. 1875.—Ed.]
- Schimper, A.F.W. [90], Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora 73: 207. 1890.
- Schultz, N. [91], Ueber die Einwirkung des Lichtes auf die Keimungsfähigkeit der Sporen der Moose, Farne und Schachtelhalme. Bot. Centralbl. Beih. 11: 81. 1901.

- Terletzki, P. [84], Anatomie der Vegetationsorgane von *Struthiopteris germanica* und *Pteris aquilina*. Jahrb. wiss. Bot. 15: 452. 1884.
- Treub, M. [84-90], Recherches sur les Lycopodiacees. Ann. Jard. Bot. Buitenzorg 4, 5, 7, 8. 1884-1890.
- True, R.H. [96]. See Kahlenberg and True [96].
- Vöchting, H. [93], Ueber den Einfluss des Lichtes auf die Gestaltung und Anlage der Blüten. Jahrb. wiss. Bot. 25: 149. 1893.
- Wheeler, H. J. [07]. See Hartwell, Wheeler and Pember [07].
- Wheeler, H.J., and Hartwell, B.L. [06], Concerning the function of sodium salts. Rhode Island Agric. Exp. Sta. Report 29, part 2: 186. 1906.
- [08], Sodium as a partial substitute for potassium. Rhode Island Agric. Exp. Sta. Report 21, part 2: 243. 1908.
- Wiegand, A. [40], Zur Entwicklungsgeschichte der Farnkräuter. Bot. Zeitg. 7: 17, 33, 49, 73, 89, 105. 1849.
- Wuist, Elizabeth D. [10], The physiological conditions for the development of monoecious prothallia in *Onoclea struthiopteris*. Bot. Gaz. 49: 216. 1910.

